

VIII. BIOSPECIMEN COLLECTION AND SHIPMENT TO GW LAB

A. Maternal Smoke Exposure

The maternal saliva collection is an easy, noninvasive procedure. You should follow these instructions in obtaining the sample. Note that the four parts of the saliva collection device described in these instructions are pictured below. Each of the four components fit inside one another. Detailed instructions for collecting the saliva sample are included in *Section VIII.B* below.



a. Stopper



b. Cotton wool swab



c. Suspended Insert



d. Centrifuge vessel

B. Steps for Collecting Maternal Saliva

- Step 1 Put on a clean pair of plastic gloves.
- Step 2 Hold the saliva collection device at the rim of the suspended insert (c) and remove the stopper (a). Removal of the stopper is easier if you slightly push it to the side when opening the suspended insert. The suspended insert will remain inside of the centrifuge vessel (d).
- Step 3 Give the device to the participant and ask her to remove the cotton wool swab (b) from it.
- Step 4 Subjects must rinse mouth and remove lipstick prior to collection.
- Step 5 Instruct the participant to chew on the cotton wool insert. She should keep the cotton in her mouth until she feels that she can no longer prevent herself from swallowing the saliva she is producing. This normally takes about 30 to 45 seconds, although it may take a little bit longer.

- Step 6 At this time, the participant should return the cotton wool swab (b) to the suspended insert (c) and return the saliva collection device to you.
- Step 7 Close the saliva collection device firmly with the stopper (a).
- Step 8 Write today's date (the date of collection) on the participant's identification label and ensure that the label is affixed to the saliva collection device.
- Step 9 Place the saliva collection device in the back pouch of the specimen bag.
- Step 10 Refrigerate the sample until taking it to the GWU lab. You will be given a portable refrigerator (that can be powered off of a car cigarette lighter) that can be used when taking the sample from the participant's home to the clinic site. Each clinic site will also have a refrigerator that can be used to store samples.
- Step 11 All biospecimen samples will be sent to the GWU lab every Wednesday at 5:00 pm and Fridays at 2:00 pm. Procedures for batching and sending urine samples to the GWU lab are discussed below.

C. Administering the Tobacco Smoke Exposure Questionnaire

Each time a saliva sample is collected from the participant, she will also need to complete the *Tobacco Smoke Exposure Questionnaire*. A copy of this questionnaire is included at the end of this chapter. This is a brief self-administered questionnaire to assess the participant's smoking status and exposure to tobacco smoke in the past seven days. Before asking the participant to complete the questionnaire, affix the subject's identification label to the questionnaire and fill in the date and site of the biospecimen collection. The questionnaire is brief and will take only a few minutes to complete.

Give the participant the questionnaire to complete on her own. If the woman seems to be having difficulty completing the questionnaire on her own, you may offer your assistance. Read each question carefully out loud, read the response categories, and then ask her which of those answers best fits her situation. Confirm her answer, and then write her response on the questionnaire. If the woman needs assistance with the questionnaire (i.e., if you end up asking her the questions), please write "interviewer-administered" at the top of this form.

Remember that a professional interviewer helps each woman feel at ease and comfortable with the interview. One key to accomplishing this goal is to be fully informed about the data collection instrument and procedures. Thus, before you begin interviewing, you should be familiar with the conventions of questionnaire design and with the content of this questionnaire.

D. Infant Urine Collection

1. Asking Mother's Assistance for Collecting Infant Urine Samples (Home Visit A)

To collect urine from the infant, you will need to ask the mother during Home Visit A to assist you with this task. Specifically, you will provide the mother with two pairs of plastic gloves, two plastic baggies (large enough to fit a baby's diaper), four diaper liners, and written instructions for using the diaper liners. You will ask the mother to use the diaper liners to collect two urine samples from the baby's diapers on the day that you plan to conduct Home Visit B. Two diaper liners will be used per diaper. It is very important that you and the mother attempt to collect urine samples from two diapers in case the first sample is contaminated with feces or baby powder or ointment. The timing of the diaper sample collection is also important since the diaper must still be wet when you arrive for the home visit, otherwise, you will not be able to extract the urine. Therefore, you must ask the mother to use the diaper liners when changing the baby's diaper about four to six hours before you arrive or first thing in the morning if the home visit is scheduled for the morning. The instructions for the mother are included in **Section D.2** below.

2. Instructions for Using Diaper Liners (for Mother)

Below are the instructions that will be given to the mother. These instructions will be available in print so that you can leave a copy with the mother. A copy of these instructions is also included at the end of this chapter.

Urine Collection Instructions For Mother

We would like to collect a urine sample from your baby's diaper on the day we come back for our second home visit. We will need samples from two diapers. To help us, we would like you to do the following on the day of our next visit.

- 1. About four to six hours before your home visit appointment, change your baby's diaper and place two diaper liners in the new diaper. If your home visit is scheduled for the morning, use the diaper liners in the first diaper you place on the baby that morning.*
- 2. Diaper liners should be placed in the area of the diaper that gets most wet. For boys, this may be more in the front of the diaper; for girl, this may be more toward the middle of the diaper. Please do not use any baby powder or ointment. Also, be sure to wear the plastic gloves while doing this so that the diaper liner is not contaminated in any way.*
- 3. The diaper liners may be attached using the adhesive strip on the liner.*
- 4. Once your baby urinates and the diaper is VERY wet, place two new liners in the new diaper. Use the second pair of plastic gloves while doing this. Please wait until the diaper is VERY wet before changing the diaper.*

5. *The old diaper with the attached diaper liners can be placed in a plastic baggie and sealed. When the second diaper is very wet, place the second used diaper in another plastic baggie and seal the bag.*
6. *We will collect both diapers and the diaper liners from you during the visit. **THANK YOU!***

The day before you visit the mother for Home Visit B, you will need to call the mother to confirm the date and time of the visit. During this call, you must remind the mother to use the diaper liners, review the instructions with her again as needed, and ask if she has any other questions.

3. Collecting the Infant's Urine Sample (Home Visit B)

As discussed above, during Home Visit A, you will have asked the mother's assistance in collecting the infant urine sample. When you arrive to the home for Home Visit B, immediately ask the mother if she has collected two wet diapers for you using the diaper liners. If she says no, ask her if the baby's diaper currently contains the two diaper liners. If she says yes, you will need to wait until the baby urinates to collect the sample. If she says no, immediately ask her to put a new diaper on the baby using two diaper liners so that you may at least obtain one sample prior to leaving the home. If necessary, you may need to come back to the participant's home later in the day to collect the sample.

If the mother has collected two wet diapers for you, thank her for her assistance. You will then need to extract the urine from the diapers using the procedures described below. Instructions for extracting urine are included in below.

Instructions for Extracting Urine from Baby's Diapers

1. Put on a pair of plastic gloves.
2. Take the cap off the urine sample cup.
3. *If using small diaper liners*, use a syringe to extract urine:
 - a. Take cap off first syringe. Remove plunger and set on clean surface.
 - b. Put the tip of the syringe in the cup.
 - c. Place first diaper liner (one diaper liner from one of the diapers with a clean sample) in the syringe (fold loosely lengthwise).
 - d. Put plunger back into syringe and push slowly.
 - e. Squeeze as hard as possible to get all of the urine into the cup until sufficient amount of urine is extracted to fill cup to maximum line.
 - f. Repeat steps a-e above for the second diaper liner as needed.
4. *If using larger diaper liners* (that don't fit into the syringe), use hands to wring urine out of the diaper liner.

- a. Fold pad in half along blue line. Hold over cup and wring diaper pad into cup thoroughly.
 - b. Unfold pad completely. Refold in other direction, and again across.
 - c. Wring again until sufficient amount of urine is extracted to fill cup to maximum line.
 - d. Repeat steps a-b with second diaper liner as needed.
5. Note: You may extract and combine urine from more than one diaper liner as long as the diapers were collected on the same day only.
 6. Cup must be filled to at least 3-4 mls (4-5 mls is ideal if possible). Cups will be marked with a minimum line. Keep in mind, more is always better!
 7. Using a pen, write the participant's ID number, date and time of collection on the label affixed to both the urine cup and plastic baggie.
 8. Place cup in plastic baggie and refrigerate until taking the sample to the GWU lab. You will be given a portable refrigerator that can be used when taking the sample from the participant's home to the GWU offices.
 9. When you return to GWU to log the home visit activities, check the sample to be sure it has been labeled properly, and place the sample into the GWU office refrigerator that will be used to store samples until they are sent to the GWU lab.
 10. NOTE: **The sample MUST be taken to the GW lab within 24 HOURS for freezing and processing!** Procedures for batching and sending urine samples to the GW lab are discussed in below.

4. Collecting Maternal Reports of Infant ETS Exposure via the Activity Diary

As discussed in *Chapter V*, in addition to collecting the infant's urine, we will assess the baby's exposure to tobacco smoke during the past seven days based on the mother's self-report. Specifically, when you return for Home Visit B and collect the infant's urine sample, you will ask the mother to refer to the *7-Day Activity Calendar* (which is given to her during Home Visit A) as you ask her specific questions to ascertain the amount of cigarettes smoked around her and her baby in various situations (e.g., in the home, away from home, etc.) This questionnaire should always be administered during Home Visit B regardless of whether or not you are able to obtain a urine sample that day. An example of a completed diary, the questions for assessing the mother's and infant's exposure to tobacco smoke, and specific instructions for completing both forms are included in *Chapter V*.

E. Sending Maternal Saliva and Infant Urine Samples to GWU Lab

1. Storing Samples

After completing a home visit, you must immediately take all completed data collection materials, including the biospecimen samples, to the GWU Healthy Infants and Mothers Program Office. Biospecimen samples will be transported to the Program Office using the portable refrigerator. Upon arrival, they will then be stored in the refrigerator at the Program Office. As mentioned above, **urine samples must be taken to the GWU lab within 24 hours for freezing.**

Maternal saliva samples can be sent at the same time or every Wednesday at 5:00 pm and Fridays at 2:00 pm.

2. Completing the Biospecimen Shipping and Results Form

Prior to taking the biospecimen samples to the GWU lab, you will need to batch the samples by type of sample and initiate a *Project DC STEP: Biospecimen Shipping and Results Form*. A copy of *Biospecimen Shipping and Results Form* is included at the end of this chapter.

The *Biospecimen Shipping and Results Form* is printed on 2-part NCR paper so that you may keep the original at the GW Program Office and the duplicate copy may be sent to the GW lab along with the samples. A separate *Biospecimen Shipping and Results Form* should be completed for each type of sample, i.e., you will need to complete one form for all maternal saliva samples and a separate form for all infant urine samples.

Once you have completed the information on the *Biospecimen Shipping and Results Form*, you may take the samples to the GW lab. All completed *Biospecimen Shipping and Results Forms* must be entered into the DMS at the end of the day. Procedures for entering this data into the DMS are included in the DMS Manual of Operations.

The following information must be completed on the *Biospecimen Shipping and Results Form* before sending the samples to the GWU lab.

1. At the top of the form, indicate the type of sample being collected: saliva, maternal urine (not applicable for the ETS home visits), or infant urine.
2. For each sample being sent to the GWU lab, you will need to write the participant's study ID, the date the specimen was collected, and the sequence number (using the codes listed at the bottom of the form). For the home visits, you will be using sequence codes 2, 3, 4 or 5 depending upon the assessment interval (i.e., baseline, 6-week postpartum, etc.)
3. After completing the above information for each sample being sent to the GW lab, indicate the date the samples are taken to the lab on the bottom right-hand corner of the form. Be sure to keep the original copy of the completed form for your own records and send the copy to the lab with the samples.

INSERT THE FOLLOWING FORMS:

Instructions for Infant Urine Collection (for Mother)

Tobacco Smoke Exposure Questionnaire

Biospecimen Shipping and Results Form